Biopotentials of Mangroves Collected from the Southwest Coast of India

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Abstract: Southwestern coast of India boasts remarkable biodiversity and presents a pristine seascape. In the present study, three mangrove species (Avicennia marina, Bruguiera cylindrica and Acanthus ilicifolius) collected from the coast were extracted in methanol and tested for different ranges of biological activities including antimicrobial activity against five species of type cultures (Microbial Type Culture Collection) of fish/shrimp Vibrio pathogens, brine shrimp cytotoxic, antifouling and ichthyotoxic activities. The overall activity profile showed that, A. marina exhibited more biopotency than B. cylindrica and A. ilicifolius. The highly active mangrove, A. marina was evaluated further to analyse the active compounds using gas chromatography. The analysis revealed a mixture of fatty acids such as alpha linolenic acid (30%), palmetic acid (21%), stearic acid (14%), lauric acid (9%), myristic acid (5%), oleic acid (5%) which might have functional role in bioactivity and can be used for the development of biodegradable antifoulants, pisicides and biopharmaceuticals.

Key words: Mangrove extract • Vibriocidal activity • Brine shrimp cytotoxicity • Antifouling activity

INTRODUCTION

Mangroves are intertidal productive forested wetland constrained to the tropical and subtropical estuarine zones, serves as a nursery, feeding and spawning ground for commercial finishes and shell fishes [1]. Habitat of mangrove plants is commonly known as mangrove swamps, tidal forests, tidal swamp forests or mangals [2]. These vascular halophytic plants constitute a vital component of marine flora and have significant ecological and socio-economic values. For centuries, mangroves have been traditionally used for food (fruits and nectar) feed and medicinal purposes in different parts of the world. They are well known to produce natural metabolites with diverse biological activities such as antibacterial [3] antiviral activity [4], antidiarrhoeal activity [5] antifeedant activity [6] insecticidal activity [7] and cytotoxic activity [8] However, during the last decade screening of mangroves for bioactive active compounds, has received high interest as a potential bioresource for novel drug leads. Until now, more than 200 bioactive metabolites have been isolated from true mangroves of tropical and subtropical populations [6]. According to their chemical structure, most of the isolated compounds belong to steroids, triterpenes, saponins, flavonoids, alkaloids, tannins and phenolics which having a wide range of therapeutic possibilities [9].

Approximately 55 species of mangroves from 22 genera were distributed in Indian ocean region [10]. The first report on regarding the chemistry of Indian mangroves was reported by Rao and Bose [11]. Recent research evidenced that Indian mangroves contained antibacterial [12], antiviral [13], mosquito larvicidal [14], antifungal [15] and antioxidant activity [16].

The biological activity of seaweeds from the southwest coast of India (Kollam coast) is already reported [17, 18]. Hitherto, mangroves of the southwest coast (Kollam coast) have not yet been studied for their biological activity. In light of this, the present study was initiated to investigate the biopotentials of mangroves from the southwest coast of India (Kollam coast) against a different range of activity including shrimp vibriocidal, brineshrimp cytotoxic, antifouling and ichthyotoxic activity.

MATERIALS AND METHODS

Collection and Extraction of Mangrove Bioactives: Three species of mangroves viz., Avicennia marina (Forsk.) (Avicenniaceae), Bruguiera cylindrica (Rhizophoraceae) and Acanthus ilicifolius (Acanthaceae) were collected and identified from mangrove forest of Ayiramthengu located in Kollam (08º54'N and 76º 38'E) area (southwest coast of India) (Fig. 1) at various dates
from April 2008 to April 2009. Prior to the extraction the leaves of respective species were cleaned, shade dried in order to prevent photolysis and thermal degradation, chopped into small pieces and ground coarsely in a mechanical grinder.

**Extraction of Bioactives:** For extraction of crude bioactives, 100 g of powdered mangrove material was refluxed three times in a 1 liter capacity round bottom flask in a water bath at 65°C for about 6 h using methanol. The extracts were filtered and concentrated to recover the excess solvents in another distillation system. The concentrated extract (about 100ml) was again filtered through a Whatman no. 1 filter paper fitted with a Buchner funnel using suction pressure. Finally, it was reduced to thick oily natured crude extract in a rotary vacuum evaporator (Yamato) at 40°C, collected in air-tight plastic vials and stored in the refrigerator for further activity studies.

**Bioassays:** Antimicrobial activity was carried out as described by Selvin and Lipton [19] against five species of type cultures (Microbial Type Culture Collection, MTCC) of fish/shrimp *Vibrio* pathogens such as *V. harveyi* (MTCC 3438), *V. alginolyticus* (MTCC 4439), *V. vulnificus* (MTCC 1145), *V. parahaemolyticus* (MTCC 451) and *V. alcaligenes* (MTCC 4442). The cytotoxic activity of mangrove extracts was tested against freshly hatched free-swimming nauplii of *Artemia salina* (Linnaeus) (*Artemia salina*, Sanders Great Salt Lake, Brine Shrimp Company L.C., U.S.A.). The assay system was prepared with 2 mL of filtered seawater containing chosen concentration of extract in cavity blocks (embryo cup) and 20 nauplii each was transferred in experimental, vehicle control and negative control wells. Invariably the concentration of the experimental systems was determined on the basis of exploratory experiments. The percentage of mortality was determined by comparing the mean surviving larvae of the test and control tubes. The LD$_{50}$ value was determined using probit scale [20]. Fingerlings (1.5-2.0 cm) of marine acclimated *Oreochromis mossambicus* were used for evaluating the ichthyotoxic potential [19]. Antifouling activity was evaluated against common rock fouler, *Patella vulgata* using ‘mollusc foot adherence bioassay’ [19]. All the experiments were performed in the present study repeated six times to validate the findings statistically.

**Gas Chromatographic Analysis of Active Mangrove, A. Marina:** The methanolic extracts of *A. marina* (10 gm) laoded on a silica gel (60-120 mesh) (E. Merck) column packed with hexane and eluted with hexane and
chloroform (9:1 to 1:9 and 100% chloroform) followed by ethyl acetate and methanol (9:1 to 1:9 and 100% methanol) to yield fourteen fractions. Individual fractions were collected and tested for bioactivity (data not shown). The fraction that was eluted using chloroform and ethyl acetate (2:8) exhibiting activity was subjected to Gas chromatography. A Gas Chromatograph (Shimadzu 2014) equipped with Flame Ionization Detector {FID} and column DB-225 (0.25×15mm) was used for the analysis.

**RESULTS AND DISCUSSION**

The plant material was subjected to an extraction process, with methanol. The yields were 3.8% for the *A. marina* extract, 3.2% for *B. cylindrical* extract and 4.2% for the *A. ilicifolius* extract.

**Antibacterial Activity:** The *in vitro* antibacterial activity revealed that the methanolic extract of mangroves had remarkable vibriocidal activity. Among the three species tested, *A. marina* exhibited wide spectrum of activity which suppress the growth of all tested vibrios, produced a mean zones of inhibition of more than 14 mm (Fig. 2). *A. ilicifolius* was found to be active against three species of vibrios such as *V. alcaligenes* (8mm), *V. vulnificus* (9 mm) and *V. alginolyticus* (10 mm) while the extract of *B. cylindrica* had the lowest activity which inhibit the growth of only two bacteria, *V. alcaligenes* (7 mm) and *V. alginolyticus* (10 mm). The difference between the antimicrobial activities of mangroves could be due to the quantity of antimicrobial substances present in each form. The sensitivity of *V. alcaligenes* to all of the mangrove extracts could be attributed due the presence of common bioactive compounds that had inhibitory effects on the microorganism.

In comparison to our study, antibacterial activity of mangroves against fish pathogens had already been studied by many authors. Abou-Elela *et al.* [21] reported the root extracts of *A. marina* had vibriocidal activity against *V. fluvialis* and *V. vulnificus*. Choudhury *et al.* [22] noted that methanolic extract of *A. cucullata* had growth inhibition against the fish pathogen, *V. alginolyticus*. Similarly, Mishra and Sree [23] reported the chloroform leaf extract of *Finlaysonia obovata* showed strong antibacterial activity against fish pathogens.

**Brine Shrimp Assay:** The brine shrimp assay is considered as a reliable indicator for the preliminary assessment of toxicity [24] and it can be extrapolated for cell line toxicity and anti-tumour activity. This assay is
Fig. 2: Antibacterial activity of *A. marina*

Fig. 3: GC profile of active column chromatography fraction of *A. marina*

widely employed in the screening process of botanical for the isolation of bioactive metabolites. In the present study, the extract of three mangrove species showed different mortality rate at different concentrations (Table 1). The mortality rate increased with the increase of concentration of each sample. The crude extracts of *A. marina* indicated the highest activity with LD₅₀ value of 318 µg/ml. The extracts of *B. cylindrica* and *A. ilicifolius* exhibited weaker brine shrimp lethality with LD₅₀ values of 410 and 475 µg/ml, respectively.

Haque *et al.*, [25] reported the cytotoxic activity of *Xylocarpus mollucensis* against brine shrimp nauplii. There are numerous reports of mangrove metabolites with cytotoxic activity [26]. Secondary metabolites which have cytotoxic activity include, diterpenoids from genus of *Bruguiera* [27]; Naphthoquinones from genus of *Avicennia* [8]; the mansonones, extracted from the heartwood of *T. populnea* [28] and alkaloids of *Alstonia macrophylla* [26]. The results implies that mangroves of the southwest coast of India contain potential bioactive compounds, which could be utilized for the development of novel anticancer leads.

**Ichthyotoxic Activity:** According to Yoshida and Ito, [29] ichthyotoxic assay is a preliminary screening method used to search for novel natural products. Natural ichthyotoxic metabolites often have a diversity of other biological activities, such as insecticidal [30] and anti-tumor [31]. Literature point out that ichthyotoxic properties of mangrove plants from different parts of the world have been discovered long ago [32-34].
In the present study, the *in vitro* ichthyotoxic activity was considered as the ability of mangrove extract to slay the fishes in respective concentration. The results showed that the mangrove extracts produced toxicity at different concentration (Table 2). At a concentration of 200 µg/ml, *A. marina* showed 100% mortality in tilapia after 4 h. The extract of *B. ceylonica* and *A. ilicifolius* exhibited 100% mortality at 250 and 300 µg/ml respectively. The mode of action of mangrove may be due to the inhibition of nervous system of fish. It was found that the mangrove extracts impart more or less same sort of behavioral changes in fishes [17]. Bandaranayake, [26] reviewed that saponins are the main factor responsible for the piscicidal activity. In recent years, a lot of work has been done on ichthyotoxic properties of mangroves, e.g. Benzoquinones embelin and 5-O-methyl embelin, from *Aegiceras corniculatum* [26], sapintoxin A from the poisonous plant *Sapium indicum* and Balanitin from *B. aegyptica* [35] exhibited strong piscidal activity. Early report envisages that Indian mangroves also had promising ichthyotoxic properties [36]. Based on the present findings, it could be inferred that mangrove extracts can be used for the management of weed and predatory fishes.

**Antifouling Activity:** Use of natural products to control fouling organisms has been studied by various people worldwide [37,38,19]. The extracts of mangroves were reported to have antifouling properties [39]. In the present study, bioactivity of mangrove extracts was based on the adherence (fouling) or shrinkage of the foot. Significant antifouling activity against *P. vulgata* was observed with methanol extract from *A. marina*, followed by extracts of *B. ceylonica* and *A. ilicifolius* (Table 3). The extract of *A. marina* exhibited complete inhibition of foot adherence/fouling at a concentration of 6 mg/ml. This concentration was considered as a safe dose, as 80% of the exposed *P. vulgata* were regained after the treatment period. However, there existed a range in mortality according to the concentration of extracts incorporated in the treatment. It was found that *A. marina* was relatively more toxic at 10 mg/ml, in which 100% mortality was occurred. Similar observations on other plant extracts have been reported [19]. These preliminary data suggest that the methanolic extract of *A. marina* may be used to develop environmental safe antifoulant to control fouling organisms.

**Gas Chromatographic Analysis of *A. Marina*:** The gas chromatography results of active column fraction revealed that the active principals were a mixture of fatty acids ranging from C-12 to C-20 (Fig.2 & Table 4). The active column fraction of *A. marina* showed six fatty acids such as alpha linolenic acid (30%), palmetic acid (21%), stearic acid (14%), lauric acid (9%), myristic acid (5%) and oliec acid (5%). Recent research reported that many fatty acids from mangroves posses antimicrobial property [40-43].

**CONCLUSION**

Mangroves from the southwest coast of India were studied for the first time for bioactivity. From the preliminary screening, we have identified mangrove plants with pronounced biological activities against limpets, brine shrimp, fishes and shrimp vibrios. Among the three species screened, the broadest activity was showed by *A. marina*, therefore this mangrove might be a potential source for developing ecologically significant bioactive compounds including biodegradable antifoulants, piscicides and biopharmaceuticals. Moreover the vibriocidal property of *A. marina* can be utilized for the development of leads for aquaculture drug development.

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